

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (currently amended) A method ~~of~~ for isolating islets from a portion of a pancreas, comprising ~~the steps of:~~

introducing ~~a pancreas or~~ the portion of a pancreas to an islet processing solution that is characterized by a plurality of process control variables;

circulating ~~an said~~ islet processing solution ~~containing a tissue dissociating compound~~ around and through the ~~pancreatic tissue~~ portion of a pancreas and past a plurality of sensors, said plurality of sensors being exposed to said islet processing solution having an output that characterizes a state of one of the process control variables;

controlling ~~one or more~~ said plurality of process control variables of the islet processing solution during islet isolation with a process controller that is in communication with said plurality of sensors, said process controller having a process control interface and being capable of changing the state of said plurality of process control variables, wherein ~~one of the one or more~~ said plurality of process control variables is include a process temperature (T), a flowrate, a

pH, a dissolved oxygen concentration, a dissolved nitric oxide concentration, an antibiotic concentration and an endotoxin concentration;

~~controlling the process temperature (T) of the islet processing solution during islet isolation between 4.0 and 44.0 degrees Celsius;~~

~~separating one or more the islets from the pancreatic tissue portion of pancreas; and~~

~~collecting the separated islets.~~

2. (currently amended) The method of claim 1, wherein ~~the process controller is~~ said controlling step comprises controlling said plurality of process control variables with a PID (proportional, integral, derivative) controller.

3. (currently amended) The method of claim 1, wherein ~~the process controller is~~ said controlling step comprises controlling said plurality of process control variables with a microprocessor temperature controller.

4. (currently amended) The method of claim 1, wherein ~~the process controller is~~ said controlling step comprises controlling said plurality of process control variables with is a microprocessor controller.

5. (currently amended) The method of claim 1, wherein ~~the process controller is~~
said controlling step comprises controlling said plurality of process control variables with a
microprocessor computer.

6. (currently amended) The method of claim 1, wherein ~~the process controller is~~
said controlling step comprises controlling said plurality of process control variables with a
variable resistance transformer.

7. (currently amended) The method of claim 1, wherein the ~~process~~ temperature is
adjusted by an electrical resistance element in thermal communication with the islet processing
solution.

8. (currently amended) The method of claim 1, wherein the ~~process~~ temperature is
adjusted by steam placed in thermal communication with the islet processing solution.

9. (currently amended) The method of claim 1, wherein the ~~process~~ temperature is
adjusted by a recirculating fluid bath in thermal communication with the islet processing
solution.

10. (currently amended) The method of claim 1, wherein the ~~process~~ temperature is adjusted by the ambient temperature of the ~~ambient surrounding~~ environment in thermal communication with the islet processing solution.

11. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process percent hydrogen ion (pH) concentration and~~ the pH is controlled by a microprocessor pH controller between pH 6.00 and pH 8.00.

12. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process percent hydrogen ion (pH) concentration and~~ the pH is controlled by a microprocessor controller between pH 6.00 and pH 8.00.

13. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process percent hydrogen ion (pH) concentration and~~ the pH is controlled by a microprocessor computer between pH 6.00 and pH 8.00.

14. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process percent hydrogen ion (pH) concentration and~~ the pH is controlled by the addition of an acid or base to the islet processing solution.

15. (cancelled) The method of claim 1, wherein a second process control variable is a process flowrate (F) and the flowrate is controlled by a microprocessor flow controller between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

16. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process flowrate (F)~~ and the flowrate is controlled by a microprocessor controller between 10.0 milliliters per minute ~~(10.0 ml/min)~~ and 4000.0 milliliters per ~~(4000.0 ml/min)~~.

17. (cancelled) The method of claim 1, wherein a second process control variable is a process flowrate (F) and the flowrate is controlled by a microprocessor computer between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

18. (cancelled) The method of claim 1, wherein a second process control variable is a process dissolved oxygen (DO) concentration and the DO concentration is controlled by a microprocessor DO controller between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and 10.0 milligrams per milliliter (10.0 mg/ml) DO.

19. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process~~ the dissolved oxygen (DO) concentration ~~and the DO concentration is~~ controlled by a microprocessor controller between 0.000000001 milligrams per milliliter ~~(0.000000001mg/ml) DO~~ and 10.0 milligrams per milliliter ~~(10.0 mg/ml) DO~~.

20. (cancelled) The method of claim 1, wherein a second process control variable is a process the dissolved oxygen (DO) concentration and the DO concentration is controlled by a microprocessor computer between 0.000000001 milligrams per milliliter (0.000000001 mg/ml) DO and 10.0 milligrams per milliliter (10.0 mg/ml) DO.

21. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process~~ the dissolved oxygen (DO) concentration ~~and the DO concentration is~~ controlled by sparging the islet processing solution with at least one inert gas ~~chosen~~ selected from the group consisting of helium, neon, argon, krypton, ~~or~~ and xenon.

22. (cancelled) The method of claim 1, wherein a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by a microprocessor NO controller between 0.000000000000001 moles per liter (0.01 picomoles/liter) NO and 1.0 mole per liter (1.0 mol/liter) NO.

23. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process~~ the dissolved nitric oxide (NO) concentration ~~and the NO concentration is~~ controlled by a microprocessor controller between 0.000000000000001 moles per liter ~~(0.01 picomoles/liter) NO~~ and 1.0 mole per liter ~~(1.0 mol/liter) NO~~.

24. (cancelled) The method of claim 1, wherein a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by a microprocessor computer between 0.000000000000001 moles per liter (0.01 picomoles/liter) NO and 1.0 mole per liter (1.0 mol/liter) NO.

25. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process~~ the dissolved nitric oxide (NO) concentration and the NO concentration is controlled by sparging the islet processing solution with at least one inert gas ~~chosen~~ selected from the group consisting of helium, neon, argon, krypton, ~~or~~ and xenon.

26. (cancelled) The method of claim 1, wherein a second process control variable is a process endotoxin (E) concentration and the endotoxin concentration is controlled by a microprocessor E controller between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

27. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process endotoxin (E) concentration~~ and the endotoxin concentration is controlled by a microprocessor controller between 0.000000001 endotoxin units units (EU) per milligram ~~(1.0 nanoEU/mg)~~ and 100.0 endotoxin units per milligram ~~(100.0 EU/mg)~~.

28. (cancelled) The method of claim 1, wherein a second process control variable is a process endotoxin (E) concentration and the endotoxin concentration is controlled by a microprocessor computer between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

29. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process endotoxin (E) concentration~~ and the endotoxin concentration is controlled by the addition of an endotoxin neutralizing protein (ENP) to the islet processing solution.

30. (currently amended) The method of claim 1 ~~and 29~~ wherein ~~a second process control variable is a process~~ the endotoxin neutralizing protein (ENP) ~~concentration and the ENP~~ concentration is controlled by a microprocessor controller between 0.000000000000001 moles per liter (0.01 picomoles/liter) ENP and 1.0 moles per liter (1.0 mol/liter) ENP.

31. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process proteolytic enzyme [PE] activity and the proteolytic~~ variables further comprise a digestive enzyme activity which is controlled by the addition of one or more antibiotics to the islet processing solution ~~chosen~~ selected from the group consisting of tetracycline, minocycline, ~~or~~ and doxycycline.

32. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process proteolytic enzyme [PE] activity and the proteolytic~~ variables further comprise a digestive enzyme activity which is controlled by the addition of one or more chelators of divalent cations to the islet processing solution ~~chosen~~ selected from the group consisting of citrate, EDTA, ~~or~~ and EGTA.

33. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process proteolytic enzyme [PE] activity and the proteolytic~~ variables further comprise a digestive enzyme activity which is controlled by the addition of one or more amino acids to the islet processing solution ~~chosen~~ selected from the group consisting of cysteine ~~or~~ and cystine.

34. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process proteolytic enzyme [PE] activity and the proteolytic~~ variables further comprise a digestive enzyme activity which is controlled by a microprocessor controller between 0.00000000000001 moles per liter (~~0.01 picomoles/liter~~) and 1.0 moles per liter (~~1.0 mol/liter~~).

35. (cancelled)

36. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process antibiotic (A) concentration and~~ the antibiotic concentration is controlled by a microprocessor controller between 0.00000000000001 moles per liter (~~0.01 picomoles/liter~~) A and 1.0 mole per liter (~~1.0 mol/liter~~) A.

37. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process~~ variables further comprise nitric oxide synthase activity (NOS) concentration and the ~~nitric oxide synthase concentration~~ which is controlled by the addition to the islet processing solution of one or more derivatives of L-arginine ~~chosen~~ selected from the group consisting of aminoguanidine, N, N'-diaminoguanidine, methylguanidine, ~~or~~ and 1, 1-dimethylguanidine.

38. (currently amended) The method of claim 1, wherein ~~a second process control variable is a process nitric oxide synthase (NOS) concentration and~~ the dissolved nitric oxide synthase concentration is controlled by the addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

39. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is an islet processing solution pressure (P) and~~ variables further comprise a the pressure which is between 1.0 pound per square inch gauge (~~psig~~) pressure and 150.0 pounds per square inch gauge (~~psig~~) pressure.

40. (currently amended) The method of claim 1, wherein ~~a second~~ said plurality of process control ~~variable is a process carbon monoxide (CO) concentration and variables~~ comprise a ~~the~~ carbon monoxide concentration which is controlled by sparging the islet processing solution with carbon monoxide.

41. (previously presented) The method of claim 1, wherein the pancreas is a human pancreas.

42. (previously presented) The method of claim 1, wherein the pancreas is a transgenic porcine pancreas.

43. (previously presented) The method of claim 1, wherein the pancreas is a non-transgenic porcine pancreas.

44. (previously presented) The method of claim 1, wherein the pancreas is a transgenic mammalian pancreas.

45. (previously presented) The method of claim 1, wherein the pancreas is a non-transgenic mammalian pancreas.

46. (previously presented) The method of claim 1, wherein the pancreas is a transgenic fish pancreas.

47-60. (canceled)

61. (currently amended) A method ~~of~~ for isolating islets from a ~~pancreas~~ pancreatic tissue, comprising ~~the steps of~~:

a step for introducing a the pancreatic tissue ~~pancreas or portion of a pancreas~~ to an islet processing solution that is characterized by a plurality of process control variables;

a step for circulating an said islet processing solution ~~containing a tissue dissociating compound around and~~ through the pancreatic tissue;

a step for controlling ~~one or more~~ said plurality of process control variables of the islet processing solution during islet isolation ~~in a predetermined manner~~, the ~~one or more~~ plurality of process control variables ~~comprises at least one chosen from the group~~ comprising: a temperature, a pH, a flowrate, a dissolved oxygen concentration, a dissolved nitric oxide concentration, a nitric oxide synthase ~~concentration~~ activity, an endotoxin concentration, an endotoxin neutralizing protein concentration, an antibiotic concentration, an amino acid concentration, a dextran concentration, a heparin concentration, ~~or and~~ a proteolytic digestive enzyme activity;

a step for separating one or more the islets from the pancreatic tissue while the one or more process control variables is are controlled; and

a step for collecting the separated islets.

62. (currently amended) The method claim 61, wherein ~~a second process control variable is a process proteolytic enzyme [PE] activity and the proteolytic digestive enzyme activity is controlled by an addition of antibiotics~~ adding an antibiotic to the islet processing solution.

63. (currently amended) The method claim 61, wherein ~~a second process control variable is a process proteolytic enzyme [PE] activity and the proteolytic digestive enzyme activity is controlled by an addition of chelators~~ adding a chelator of divalent cations to the islet processing solution.

64. (currently amended) The method of claim 61, wherein ~~a second process control variable is a process proteolytic enzyme [PE] activity and the proteolytic digestive enzyme activity is controlled by an addition of amino acids~~ adding an amino acid to the islet processing solution.

65. (currently amended) The method of claim 61, wherein ~~said a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by an addition~~ adding to the islet processing solution of one or more derivatives of L-arginine ~~chosen~~ selected from the group consisting of aminoguanidine, N, N'-diaminoguanidine, methylguanidine, ~~or~~ and 1, 1-dimethylguanidine.

66. (currently amended) The method of claim 61, wherein ~~said a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is~~ controlled or inhibited by ~~an addition of~~ adding 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

67. (currently amended) The method of claim 61, wherein ~~said a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is~~ controlled or inhibited by ~~an addition of amino acids~~ adding an amino acid to the islet processing solution.

68. (currently amended) The method of claim 61, wherein ~~said a second process control variable is a process dissolved nitric oxide (NO) concentration and the NO concentration is~~ controlled or inhibited by ~~an addition of~~ adding one or more of the following to the islet processing solution ~~chosen from~~ one or more of the compounds selected from the group consisting of dextran or and heparin.

69. (currently amended) The method of claim 61, wherein ~~said a second process control variable is process dissolved nitric oxide (NO) concentration and the NO concentration is controlled~~ or inhibited by ~~an addition~~ adding one or more antibiotics to the islet processing solution ~~chosen~~ one or more antibiotics selected from the group consisting of tetracycline, minocycline, or and doxycycline.

70. (currently amended) The method of claim 61, wherein a ~~second process control variable is a process~~ the nitric oxide synthase activity (NOS) concentration and the NOS concentration is controlled or inhibited by ~~an addition of~~ adding one or more antibiotics to the islet processing solution ~~chosen~~ one or more antibiotics selected from the group consisting of tetracycline, minocycline, ~~or~~ and doxycycline.

71. (cancelled) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the endotoxin concentration in the islet processing solution.

72. (cancelled) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the nitric oxide concentration in the islet processing solution.

73. (new) The method of claim 61 wherein:

said step for controlling one or more of said plurality of process control variables is accomplished with a process controller.

74. (new) The method of claim 61 wherein:

one or more of said plurality of process control variables is controlled in said step for controlling.

75. (new) The method of claim 61 wherein:

one or more of said plurality of process control variables is controlled with a process controller in said step for controlling.

76. (new) An apparatus for isolating islets from a pancreatic tissue, said apparatus comprising:

a heated feed tank;

an reactor for containing a physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature, a pressure, an endotoxin concentration and a dissolved carbon dioxide concentration, said reactor comprising a flow loop that connects a digestion chamber, a sparging vessel and a process pump, said flow loop being connected through a first valve to said heated feed tank, said process pump being operative to circulate said physiologic process solution through said reactor;

a process controller;

a sensor block that is located on said flow loop upstream from said digestion chamber, said sensor block having a plurality of sensors that send signals to said process controller, said sensor block comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, a pressure sensor for sensing said pressure, an endotoxin sensor assembly for sensing said endotoxin concentration, and a carbon dioxide sensor for sensing said dissolved carbon dioxide concentration;

a process cooler that is located on said flow loop and a process heater that is located on said flow loop;

a plurality of solution pumps comprising a digestive enzyme solution pump for pumping a digestive enzyme solution from a digestive enzyme solution reservoir into said flow loop, an endotoxin neutralizing protein solution pump for pumping an endotoxin neutralizing protein solution from an endotoxin neutralizing protein solution reservoir into said flow loop, an acid solution pump for pumping an acid solution from an acid solution reservoir into said flow loop, and a base solution pump for pumping a base solution from a base solution reservoir into said flow loop;

a source of oxygen gas controlled by a oxygen gas valve for introducing a stream of oxygen gas into said sparging vessel, a source of carbon dioxide gas controlled by a carbon dioxide gas valve for introducing a stream of carbon dioxide gas into said sparging vessel, and a source of an inert gas controlled by an inert gas valve for introducing a stream of inert gas into said sparging vessel; and

an auto-collector that is connected to said flow loop for collecting the islets;

wherein said process controller is operative to compare the dissolved oxygen concentration in said physiologic process solution to a dissolved oxygen setpoint and activate either said oxygen gas valve to add dissolved oxygen or said inert gas valve to remove dissolved oxygen, to compare said dissolved carbon dioxide concentration to a dissolved carbon dioxide setpoint and activate said inert gas valve to remove dissolved carbon oxide, to compare said dissolved nitric oxide concentration to a dissolved nitric oxide setpoint and activate said inert gas valve to remove dissolved oxygen and inhibit nitric oxide formation, to compare said pH to a pH setpoint and activate either said acid solution pump to reduce said pH or said base solution pump

to increase said pH, to compare said temperature to a temperature setpoint and to activate either said process cooler to decrease said temperature or said process heater to increase said temperature, to compare said endotoxin concentration to an endotoxin concentration setpoint and activate said endotoxin neutralizing protein solution pump and to compare said dissolved carbon dioxide concentration to a dissolved carbon dioxide concentration setpoint and increase said dissolved carbon dioxide concentration by introducing said stream of carbon dioxide gas into said sparging vessel.

77. (new) The apparatus of claim 76 wherein said plurality of solution pumps further comprise a proteolytic control means selected from the group consisting of an amino acid solution pump for pumping an amino acid solution from an amino acid solution reservoir into said flow loop and a chelator solution pump for pumping a chelator solution from an chelator solution reservoir into said flow loop; and wherein said process controller is operative to compare said proteolytic enzyme activity to a proteolytic enzyme activity set point and activate either said amino acid pump or said chelator solution pump.

78. (new) The apparatus of claim 76 wherein said plurality of solution pumps further comprise a dissolved nitric oxide control means selected from the group consisting of an antibiotic solution pump for pumping an antibiotic solution from an antibiotic solution reservoir into said flow loop and a dextran or heparin solution pump for pumping a dextran solution or a heparin solution from a dextran solution or a heparin solution reservoir into said flow loop; and wherein said process controller is operative to compare said dissolved nitric oxide concentration

to a dissolved nitric oxide concentration set point and activate either said antibiotic solution pump or said dextran or heparin solution pump.

79. (new) The apparatus of claim 76 wherein moving means are provided that are operative to rotate, move linearly or move eccentrically said digestion chamber, valve means are provided that are operative to circulate the physiologic process solution through said digestion chamber in a forward direction and in a reverse direction and transducer means are provided that are operative to cause sonication of the physiologic process solution circulating through said digestion chamber.

80. (new) A method for isolating islets from pancreatic tissue, said method comprising:
filling the reactor of claim 76 with a first portion of said physiologic process solution;
circulating said first portion of said physiologic process solution through said reactor;
draining said first portion of said physiologic process solution from said reactor to
produce a rinsed reactor;

refilling said reactor with a second portion of said physiologic process solution by
circulating said second portion of said physiologic process solution through said reactor;
pausing the circulation of said second portion of said physiologic process solution
through said reactor;

adding the pancreatic tissue to said digestion chamber;

restarting the circulation of said second portion of said physiologic process solution
through said reactor and performing real-time data acquisition by said plurality of sensors;

sampling said second portion of said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said second portion of said physiologic process solution;

when the islets have been liberated from the pancreatic tissue into said second portion of said physiologic process solution, cycling said reactor to affect dilution and collection of the islets.

81. (new) The method of claim 80 wherein circulation of said second portion of said physiologic process solution through said reactor comprises:

flowing said second portion of said physiologic process solution through said reactor in a forward direction; and/or

flowing said second portion of said physiologic process solution through said reactor in a reverse direction.

82. (new) A method for isolating islets from pancreatic tissue, said method comprising: circulating a physiologic process solution through a reactor having a flow loop that connects a digestion chamber into which said pancreatic tissue has been deposited, a sparging vessel and a process pump, said physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature and an endotoxin concentration;

performing real-time data acquisition by means of a plurality of sensors that are exposed to physiologic process solution as it circulates through said flow loop, said plurality of sensors comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a

dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, and an endotoxin sensor for sensing said endotoxin concentration;

automatically controlling said dissolved oxygen concentration, said dissolved nitric oxide concentration, said pH, said temperature and said endotoxin concentration;

sampling said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said physiologic process solution;

when the islets have been liberated from the pancreatic tissue into said physiologic process solution, collecting the islets.

83. (new) An apparatus for isolating islets from pancreatic tissue, said apparatus comprising:

means for circulating a physiologic process solution through a reactor having a flow loop that connects a digestion chamber into which said pancreatic tissue has been deposited, a sparging vessel and a process pump, said physiologic process solution having a dissolved oxygen concentration, a dissolved nitric oxide concentration, a pH, a temperature and an endotoxin concentration;

means for performing real-time data acquisition by means of a plurality of sensors that are exposed to physiologic process solution as it circulates through said flow loop, said plurality of sensors comprising a dissolved oxygen sensor for sensing said dissolved oxygen concentration, a dissolved nitric oxide sensor for sensing said dissolved nitric oxide concentration, a pH sensor for sensing said pH, a temperature sensor for sensing said temperature, and an endotoxin sensor for sensing said endotoxin concentration;

means for automatically controlling said dissolved oxygen concentration, said dissolved nitric oxide concentration, said pH, said temperature and said endotoxin concentration;

means for sampling said physiologic process solution to determine whether the islets that have been liberated from the pancreatic tissue into said physiologic process solution;

means for collecting the islets when the islets have been liberated from the pancreatic tissue into said physiologic process solution.